Submitted Abstract Data

Please note that after submitting your abstract data, by clicking the "submit" button below, you will not be able to make any changes.

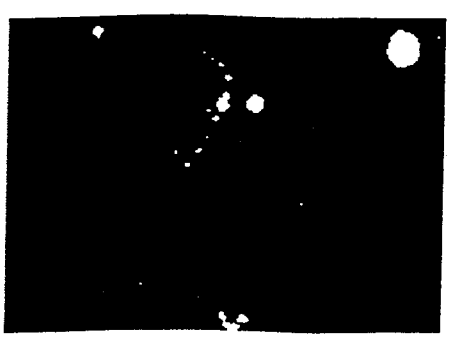
abstract nr.: 960

Detection of Naphthalene Dioxygenase mRNA Transcripts by Fluorescent in situ Hybridization (FISH) with Tyramide Signal Amplification (TSA)

Author: Bakermans, C , (
Co-author(s): Madsen, E.L. , Cornell University, Ithaca, NY, United States of America
Topic: Microbial aspects of bioremediation
Keywords: fluorescent in situ hybridization, microbial activity, naphthalene, biodegradation

The detection and quantification of in situ microbial activity remains an elusive goal for microbiologists and remains hindered by available methodologies. In this study, a TSA-FISH method was developed to detect naphthalene dioxygenase (nahAc) mRNA to determine the relevance of model nahAc alleles to activity at a coal tar contaminated site. In TSA, a biotin labeled probe is detected when streptavidin conjugated horseradish peroxidase catalyzes localized deposition of fluorescently labeled tyramide onto electron-rich moieties. Detection sensitivity was further increased by using a Hamamatsu CCD camera and extended exposure times. Pseudomonas putida NCIB 9816-4 was used to refine the protocol. Probe-conferred fluorescence was detected only when nahAc expression was induced by salicylic acid. RNase treatment eliminated detection of probe-conferred fluorescence, while DNase treatment enhanced fluorescence. TSA-FISH is implemented without any post-sampling incubation steps that may alter the composition or activity of naturally occurring microbial communities. When applied to contaminated groundwater samples, probe-conferred fluorescence was observed in a subset of the cells present (see Figures). By identifying mRNA transcripts present in cells fixed immediately from environmental samples, TSA-FISH has the potential to document in situ expression of specific genes.

DAPI fluorescence of groundwater community.



CY3 (probe conferred) fluorescence of same field.

Presentation Preference: Poster Presentation